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Behavioral and Physiological Changes Produced by a Supralethal Dose of Ionizing Radiation: Evidence for Hormone-Influenced Sex Differences in the Rat[1]

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A sufficiently large and rapid dose of ionizing radiation produces an immediate but transient behavioral incapacitation. Acute hypotension often accompanies the disorder. Although the etiology of this syndrome is unclear, it has been suggested that an increase in histamine excretion contributes to it. Since histamine is known to interact with the endocrine system and since estrogens have been shown to prolong the life of animals exposed to potentially lethal doses of radiation, it was also hypothesized that females might be relatively less affected by an acute, large dose of ionizing radiation. Male and female rats were trained on an avoidance task, irradiated, and then retested. Females showed a less severe decrement after radiation exposure than males. Likewise, females did not suffer the severe hypotension normally associated with male radiogenic early transient incapacitation (ETI); rather, an acute hypertension was produced in females. A second series of experiments revealed that differences in male and female radiation response were eliminated by gonadectomy. Systemic estradiol injections produced strikingly feminine (i.e., superior) postirradiation avoidance responses as well as hypertension in neutered rats. Testosterone injections had no effect on either measure. Central nervous system alterations have been correlated with the ETI. Therefore, final experiments sought a possible central locus of the action of estradiol. It was found that exposure of the nucleus preopticus medialis to estrogens produces postirradiation benefits in avoidance performance and blood pressure similar to those seen after systemic estradiol treatments. Nucleus amygdaloideus medialis implants produced no such benefits.

INTRODUCTION

Rats that have been exposed to a sufficiently large and rapid dose of ionizing radiation exhibit an early transient incapacitation (ETI) which is characterized

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by akinesia (*1*) and decrements in motivated (avoidance) behaviors (*2*). Typically, performance on a behavioral task is compromised for a period of up to 0.5 hr after the irradiation. This behavioral decrement is followed by temporary recovery, progressively poorer performance, permanent incapacitation, and death. The syndrome has also been noted in pigs (*3*) and monkeys (*4*) trained in a shock-avoidance task.

Although the ETI syndrome was identified years ago, its exact etiology is still quite uncertain. One specific hypothesis is that the accumulation of the biogenic amine, histamine, may contribute to the ETI. In this regard Doyle and Strike (*5*) demonstrated an increase in blood histamine after 4000 rad of ionizing radiation. This increase peaked at about 3 min postirradiation, decayed with time, and approached control values at 20 min (about when the subject's behavior returned to preirradiation baseline). Blood pressure is one physiological factor that is sensitive to histamine changes, and several investigators (*6*, *7*) have demonstrated that, although it is not the sole cause of behavioral ETI (*8*), severe peripheral hypotension often accompanies periods of postirradiation performance decrement. Pretreatment with the antihistamine, chlorpheniramine maleate, effectively alleviates the hypotension associated with ETI and may improve performance (*9*).

Whether histamine changes can fully account for behavioral and cardiovascular anomalies is still in question. This paper will not attempt to address the histamine hypothesis directly. Instead, the present investigation is motivated by a known interaction between histamine and gonadal hormones. Urinary histamine excretion is increased significantly by estradiol injections (*10*), and female rats excrete six times as much free histamine as males (*11*). This histamine/estrogen interdependency may be of interest to radiobiologists since estradiol pretreatment of X-irradiated mice prolongs the life of some mice and reduces death rates (*12–15*). Conceivably the presence of estrogens may also play a part in *acute* radiation phenomena such as ETI, and males and females might show different kinds of responses to a radiation insult. To test this notion, males and females were compared on a learned avoidance task postirradiation. Since the blood pressure drops in male rats following large, rapid doses of ionizing radiation (*16*), this correlate of the ETI syndrome was also measured in rats of both sexes. It is reported here that female radiogenic behavioral incapacitation was less severe than that experienced by males and that females were mildly hypertensive in this time period immediately after irradiation.

In nonirradiated animals, gonadal hormone injections have previously been shown to alter the performance of avoidance tasks. Intact adult females injected with a single dose of testosterone produced rats whose acquisition of an avoidance task was dramatically masculinized (i.e., inferior). Indeed, the performance of these animals was actually poorer than that of normal males (an effect that may be dependent on the relatively high dose of androgen used) (*17*). Estrogens may also play a key role in the facilitation of the conditioned avoidance response. Avoidance tasks are performed more efficiently (fewer errors and more quickly) by female rats at times during the estrus cycle when their estradiol levels are highest (*18*).

Gonadal hormones have also been shown to alter various cardiovascular

measures (*19*). Specifically, estrogens may be important in maintaining vascular tone and blood pressure during circulatory stress (*20*). Altura (*21*) has shown, for instance, that inbred female rats were more resistant to the lethal effects of two different circulatory stresses than were inbred male animals of the same strain. Pretreatment of male rats with single or multiple doses of estradiol (a naturally occurring estrogen in rats and man) effectively enhanced the male cardiovasculature's resistance to stress. Thus female rats and males treated with estradiol maintained near-normal blood pressures and venular tone when subjected to lethal circulatory stress. These animals also sustained a more reactive vasculature than usually found in untreated males (*21–23*).

As was noted earlier, estrogen treatments permitted a greater survival rate in male mice irradiated with potentially lethal doses. Castration of the mice also had a beneficial effect on survival. However, castrated mice that received a single injection of estradiol benzoate (*24*) experienced the fewest fatalities.

Here, a second series of studies investigated the effect of gonadectomy and treatment with specific gonadal hormones on the acute performance and blood pressure responses of rats exposed to a large dose of ionizing radiation. This research suggested that estradiol may be an important factor in the female rats' apparent radioresistance.

A final set of experiments addressed the question of whether estradiol's beneficial effects are produced by direct action on the central nervous system (CNS). There presently exists evidence that CNS damage indeed plays a critical role in the production of ETI symptoms. EEG changes accompany the known behavioral decrements seen postirradiation (*25*, *26*). When the head had been shielded, dogs (*27*) and minature pigs (*28*) performed well with no incapacitation for several hours after receiving extremely high supralethal doses of pulsed mixed γ–neutron radiation. Likewise, irradiation of the heads of dogs and pigs (trunk shielded) caused the same type of incapacitation as in unshielded subjects that had comparable whole-body doses.

The reduction of aortic blood pressure after a high-radiation dose could also conceivably be the result of a derangement of CNS function. Montgomery and Warren (*29*) supported this hypothesis with their finding that spine-transected rats failed to exhibit hypotension after radiation insult. These data suggest that CNS damage is critical in the production of behavioral incapacitation and blood pressure decline.

However, others have shown that exposure of the trunk of head-shielded monkeys produces decrements in avoidance conditioning. Although these deficits are less severe than those experienced by head-exposed animals, the data suggest a peripheral component of the ETI (*30*). Likewise, Chapman and Young (*31*) hypothesized that the transient behavioral symptoms, hypotension, and decreased cerebral blood flow observed after whole-body irradiation are not due primarily to a direct effect of radiation on the CNS. Rather, they see peripheral vascular resistance and permeability changes as the critical cause of hypotension and concurrent behavioral difficulties (*31*, *32*).

Other work indicates that there is a topographical distribution of estrogen-containing cells in the brain of several species (*33–36*). It is possible that these

cells, which are prevalent in the nucleus preopticus medialis (MPO) and the nucleus amygdaloideus medialis (MA), may be the sites of action of estrogen's apparent radioprotective effects. The last studies presented here investigated how intracranial hormones alter the behavior and blood pressure of irradiated gonadectomized rats.

MATERIALS AND METHODS

Experimental Groups

Experimentally naive Sprague–Dawley rats (200–300 g) were used in these experiments. At the time of irradiation the ages (about 10 weeks) of animals in separate groups were not significantly different (Mann–Whitney U test, $P > 0.05$) (*37*). Rats were housed three to a cage in a room illuminated from 7:30 AM to 4:00 PM daily. Ambient temperature was set at 20°C. Purina Rat Chow and water were continuously available. Three sets of experiments were conducted (see Table I): (a) Intact male and female comparison (sex dichotomy), (b) gonadectomized rat comparison with and without systemic gonadal hormone

TABLE I

Experimental Groups and Conditions

Experiment	*Sex of subject*	*Number of subjects*		*Surgery*[a]	*Hormone manipulation*[b]
		Avoidance	*Blood pressure*		
Sex dichotomy	Male	6	6	None	None
	Female	6	6	None	None
Systemic hormone manipulation	Male	6	—	None	None
	Male	6	6	CAST	Sham
	Male	6	6	CAST	EST
	Male	6	6	CAST	TEST
	Female	6	—	None	None
	Female	6	6	OVX	Sham
	Female	6	6	OVX	EST
	Female	6	6	OVX	TEST
Intracranial hormone manipulation	Male	6	6	CAST + MPO	Sham
	Male	6	6	CAST + MPO	EST
	Male	6	6	CAST + MA	Sham
	Male	6	6	CAST + MA	EST
	Female	6	6	OVX + MPO	Sham
	Female	6	6	OVX + MPO	EST
	Female	6	6	OVX + MA	Sham
	Female	6	6	OVX + MA	EST

[a] CAST = castration; OVX = ovariectomy; MPO = implantation of cannulas in the nucleus preopticus medialis; MA = implantation of cannulas in the nucleus amygdaloideus medialis.

[b] Sham = oil (vehicle) injections (systemic manipulation) or cholesterol implantation (intracranial manipulation); EST = estradiol benzoate; TEST = testosterone propionate.

replacement (systemic hormone manipulation), and (c) gonadectomized rat comparison with and without intracranial estrogen replacement (intracranial hormone manipulation). Half of the rats in each of the above experiments were trained on an avoidance task. Blood pressures were determined for the other half of the subjects (see Table I).

Avoidance Performance

Rats were trained to avoid foot shock by leaping a distance of 10 cm from the floor of a cage up onto a retractable Lucite ledge. The avoidance apparatus consisted of a Plexiglas chamber 37 × 24 × 37 cm in height with a grid floor that could be electrified with scrambled shock. A signal click preceded the foot shock by 5 sec. The Lucite ledge was moved into the cage at the onset of the click and retracted after the foot shock (0.5 mA for 10 sec). A microswitch on the ledge indicated if the subject successfully moved up to the shelf before the shock. The intertrial interval was held constant at 30 sec.

Subjects were trained to meet an avoidance-response criterion of 100% over 50 consecutive trials. Rats received at least 50 trials once a day until this criterion was met; this usually took about 5 days. The subjects were then irradiated. Immediately after irradiation, 50 trials were again administered in the avoidance chamber.

Blood Pressure Determinations

Systolic blood pressure was measured indirectly by using Narco Bio-System's occluding tail cuff (32 mm long, 13 mm inside diameter), pneumatic pulse transducer, and programmed electro-sphygmomanometer (ES). Blood pressure readings were obtained while rats were confined in a ventilated transparent plastic animal housing which had a built-in warming element (used to raise the environmental temperature of the subject).

The indirect blood pressure-recording technique for rats involves the occlusion of circulation in the tail with an annular cuff and detection of the pulse as the cuff pressure is lowered. The pressure at which the first pulse reappears is a measure of the systolic blood pressure of the animal. Obtaining such a blood pressure reading may be difficult since a pulse in the tail will often be absent (unless the animal's environmental temperature is raised). Since the tail of the rat is an essential part of its heat-exchange mechanism, circulation may be cut off in the tail if the animal is cool (*38*). By prewarming the environment, circulation can be easily promoted and maintained in the tail to permit use of this occluding technique for blood pressure measurement. The principle does not necessarily involve raising the temperature of the animal but merely supplying slightly more heat than the animal can normally dissipate through respiration and normal body circulation. When this point is reached, the animal must use his tail as an additional heat-dissipating system by promoting circulation in it.

Each rat was constrained in the animal housing with its tail protruding outside (see Fig. 1). The metal tubular cuff was slipped onto the tail as far as it would go and then connected to the pump in the ES. Tubing from the pneumatic pulse

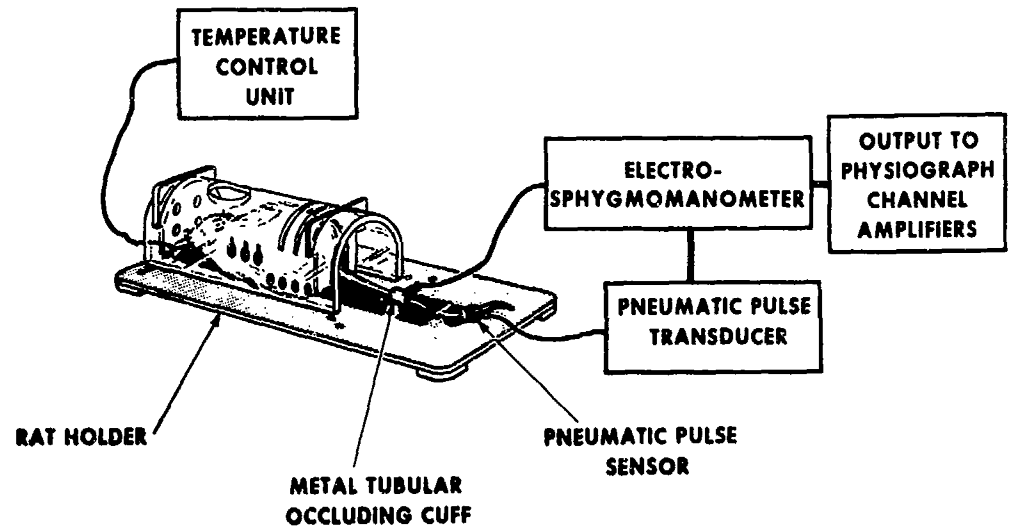

FIG. 1. Schematic representation of apparatus used to measure blood pressure (indirect method).

transducer was taped lengthwise over the animal's tail 3 cm distal to the cuff and then also connected to the ES. The output of the ES was reproduced on a physiograph recorder. Each rat was warmed until a pulse could be detected in the tail. Care was taken to not supply too much heat, which would cause behavioral signs of apparent discomfort. When detectable tail circulation had been established, the cuff was inflated to 200 mm Hg at a rate of 20 mm Hg/sec. As the cuff slowly deflated, the systolic pressure was noted as the point at which pulsations first appeared on the descending pressure curve. This method of indirect systolic blood pressure measurement has been shown to correlate highly ($r = 0.974$, $P < 0.001$) with direct methods using indwelling carotid catheters (*38*). A baseline systolic pressure reading was taken immediately before irradiation. Subsequent measures were made at 5, 10, 15, and 20 min postirradiation.

Irradiation Procedure

The Armed Forces Radiobiology Research Institute Linear Accelerator was used to expose rats to high-energy electron radiation. Each animal was irradiated individually with its right side to the source while in a constraining polyethylene tube. Electrons were accelerated to an energy of 18.1 MeV at a peak beam current of 0.44 amperes. Pulse duration was 4 μsec, and pulses were delivered at a rate of 15/sec. In order to make the field more uniform, a water-scatter device was used which lowered the energy of the beam by 3.5 MeV. Each rat was positioned 3.5 m from the source and received a midline tissue dose of 10,000 rad (13 rad/pulse), which has been demonstrated to consistently produce an ETI (*39*). Total exposure time was between 0.8 and 1.2 min. Dosimetry was accomplished by using 0.05-cm^3 tissue-equivalent ion chambers whose calibration is traceable to the National Bureau of Standards. Animals were retrieved within 1 min after exposure. Previously published data indicate that constraint and sham irradiation do not alter performance on the avoidance task (*39*). Nor does sham irradiation alter the blood pressure responses of either sex (pilot data).

Surgical and Hormone-Replacement Procedures

Gonadectomy. Male and female rats were gonadectomized under ether anesthesia within the first 5 days after birth. Early gonadectomy almost certainly eliminates most adult sexual behavior and makes hormone replacement therapy most effective (*40*). The rats were raised in an environment similar to that described in the previous experiments. When the rats had achieved a weight of 200 to 300 g, systemic hormone or intracranial hormone manipulations were begun.

Systemic hormone injections. For 7 days before irradiation, selected subjects (see Table I) received daily subcutaneous (sc) injections of 1 μg estradiol benzoate dissolved in 0.25 ml olive oil. Other subjects received daily sc injections of 500 μg testosterone propionate (in 0.25 ml olive oil) for a week before irradiation. These doses of gonadal hormones have been shown to restore adult sexual behaviors in gonadectomized rats (*41*, *42*). Additional rats were injected with 0.25 ml olive oil sc once a day for 7 days before irradiation (sham hormone injection). On the last injection day, all rats were irradiated in the manner described above. Pilot data indicate that these steroid and vehicle-control injections did not alter the rat's ability to perform the avoidance task.

Intracranial hormone injections. When 96 gonadectomized rats (see Table I) had achieved a weight of 200 to 300 g, they were implanted with chronic bilateral intracranial cannulas. Each cannula contained either 50 μg of crystalline estradiol benzoate or 50 μg of cholesterol benzoate (sham control). These cannulas were aimed at either the nucleus preopticus medialis (MPO) (0.5 mm posterior to bregma, ±1 mm lateral to midline and 8.0 mm below the skull) (*43*) or the nucleus amygdaloidus medialis (MA) (2 mm posterior to bregma, ±3.3 lateral to midline and 8.0 mm below the skull) (*43*).

Carrier cannulas were constructed from 20-gauge stainless-steel hypodermic tubing using the methods of Smith *et al.* (*44*). They were chronically implanted in rats anesthetized with 6.5 mg/kg xylazine and 80 mg/kg ketamine HCl. Atropine sulfate (0.4 mg/kg) was used as a preanesthetic agent. Steroids were ejected from the cannulas at the end of surgery. Animals were allowed 1 week to recover from surgery before they were irradiated. All cannula placements were histologically verified at the end of the experiments. Pilot data indicated that this surgical procedure did not alter the rats' ability to perform the avoidance task.

RESULTS

Sex Dichotomy Experiments

Avoidance Task

Intact male rats exhibited a more profound and longer-lasting decrement in avoidance response than did intact female rats. Figure 2 illustrates this effect. The mean percentage avoidance for each group of animals is shown for eight 2.5-min periods (five trials each) following irradiation. Close inspection of individual animal scores at specific time periods suggests that they are not drawn from a normal distribution. For this reason, nonparametric statistical comparisons were made. Clearly, compared to their 100% baselines, both males

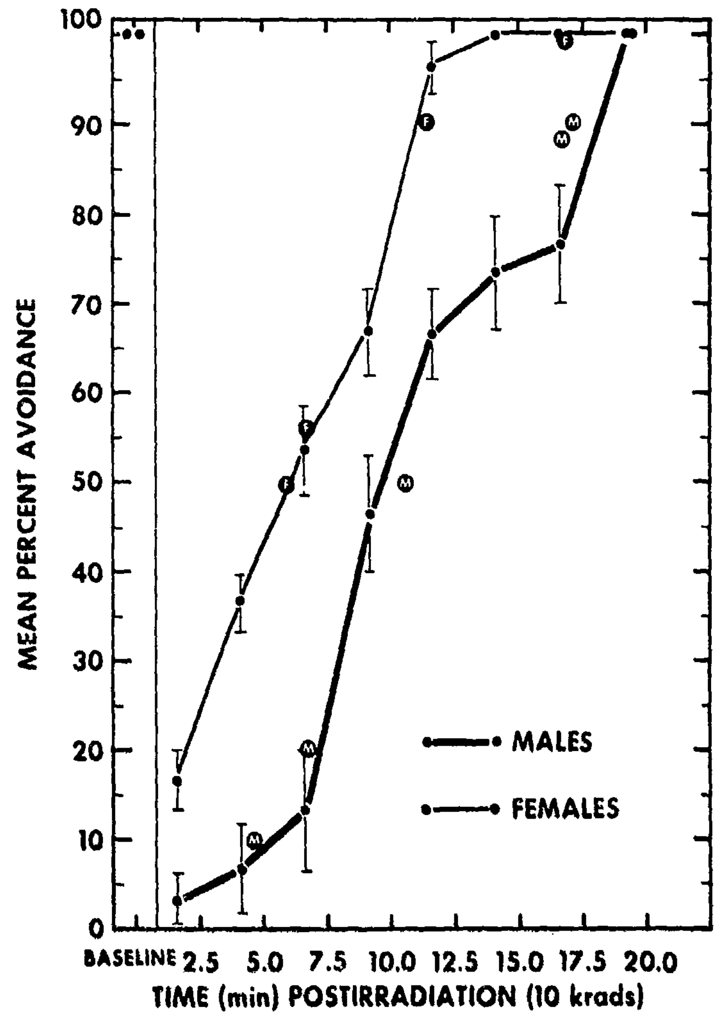

FIG. 2. Mean percentage avoidance of both sexes as a function of time postirradiation. After irradiation, avoidance performance decreased markedly. However, female rats consistently avoided foot shock more often and recovered to baseline rates of performance more quickly than males. Probit curve fitting provides interpolated data points for both males (M) and females (F) that closely approximate the actual empirical data. Variance indicators are the standard errors of the means.

and females showed a large decline in their ability to move up to the safe shelf before administration of shock. A Mann–Whitney U comparison (*37*) between the percentage-avoidance scores of the two sexes in the first 2.5 min reveals a significant sex difference ($P = 0.008$), as do similar comparisons at 7.5 min ($P = 0.001$) and 17.5 min ($P = 0.002$). Females performed significantly better than males in the time immediately after irradiation. Likewise, female performance was always above that of the male until the 20-min time period. Here the two functions met as both sexes returned to their baseline levels of responding.

It is apparent from inspection of Fig. 2 that females not only performed better at all time periods, but that they also recovered much more quickly. It is possible to compute the amount of time it takes each animal to achieve 90% avoidance response by fitting a smooth function to the data and then interpolating the 90% value from this curve. The probit function seems to offer a reasonable fit to the raw data (see fitted points in Fig. 2). This may not be too surprising since the

probit form is often used to describe that of cumulative records. In the present situation, cumulative benefits gained by the passage of time may account for the oogive shape of the male and female data curves. Curve fitting offers another practical benefit here. Since each 2.5-min period contains five trials, the percentage avoidance statistic offers only increments of 20%. Thus, many ties are likely, since only six percentage outcomes are permitted (0, 20, 40, 60, 80, 100%). Interpolation using the probit curve allows ranking of these scores.

The time to reach 90% was derived for each animal, and a Mann–Whitney *U* was computed, comparing the two groups. On the average it took males 17.5 min to recover whereas females were back up to 90% of baseline performance after only 12 min. There was no overlap between the individual times of the two groups, and they differed significantly ($P = 0.001$).

Blood Pressure

Intact male and female rats exhibited different kinds of blood pressure responses after irradiation. Figure 3 presents two sets of raw records that illustrate this finding. The male record indicates a replication of the usual finding (*16*) of profound hypotension following large doses of ionizing radiation. The female rat, however, exhibited a hypertension in this same time period. The grouped data for all animals also clearly demonstrate this sex difference (see Fig. 4).

The distribution of raw blood pressure readings obtained in this study was not a normal one. Thus, since the data approach normality if they are transformed into logarithms, all measurements underwent a log conversion prior to a parametric statistical treatment. The difference was computed between these log-converted blood pressure measures taken postirradiation and the log-converted preirradiation baseline. The mean of these four scores was derived for each animal. A *t*

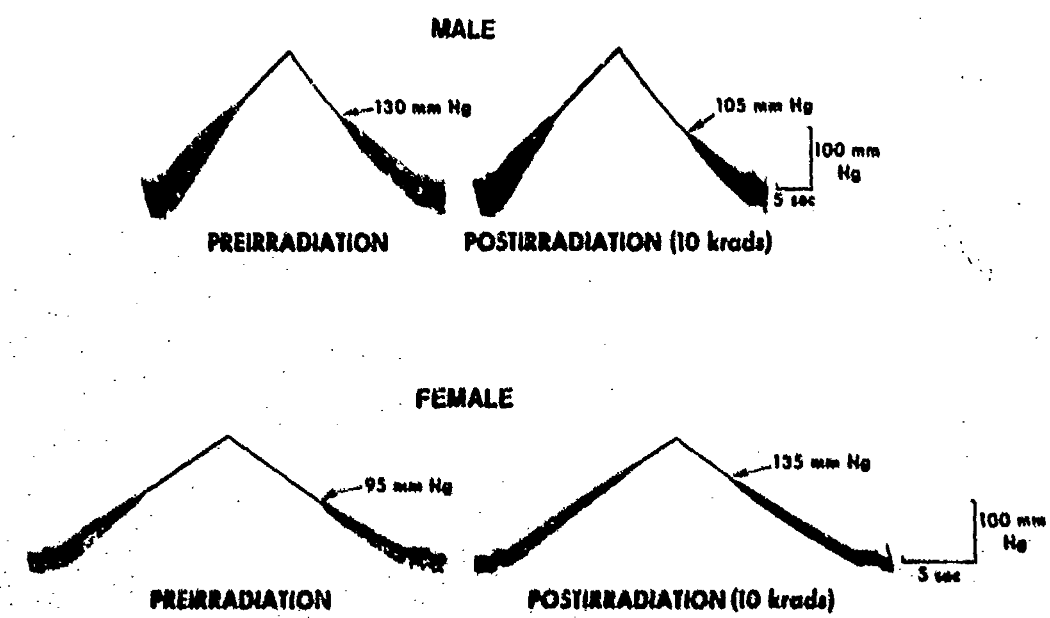

FIG. 3. Individual raw blood pressure recordings of a male rat and a female rat indicate that male systolic pressure declines substantially after exposure to ionizing radiation, and females show a hypertension in the same time period.

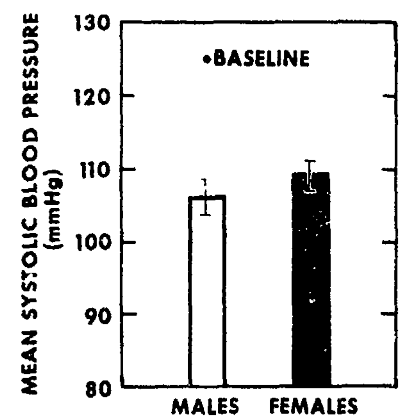

FIG. 4. Baseline and postirradiation systolic blood pressure readings of both sexes. While male (●) and female (○) perirradiation baseline blood pressures differ quite markedly, the postirradiation systolic blood pressures (columns) are similar. Variance indicators are the standard error of the mean.

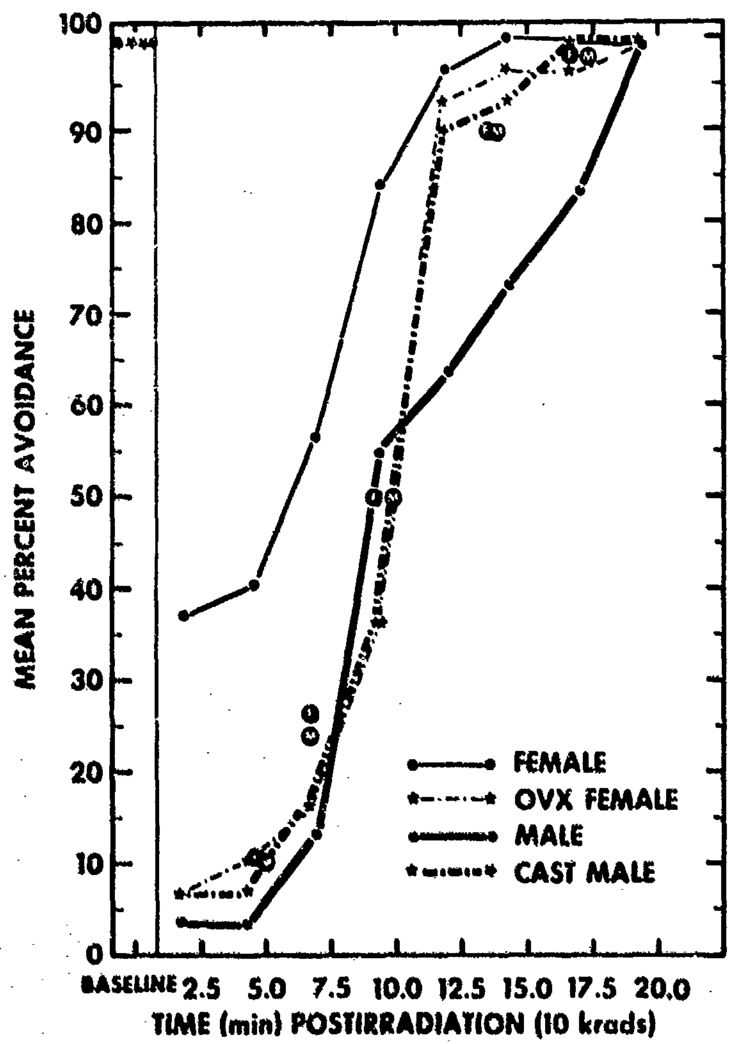

FIG. 5. Mean percentage avoidance as a function of time postirradiation. Gonadectomy (OVX = ovariectomy, CAST = castration) eliminates the differences observed between intact male rats and female rats. Neutered rats of both sexes suffer initial decrements as severe as intact males but recover quickly, like intact females. Probit curve fitting provides interpolated data points for both castrated males (M) and ovariectomized females (F) which closely approximate the empirical data.

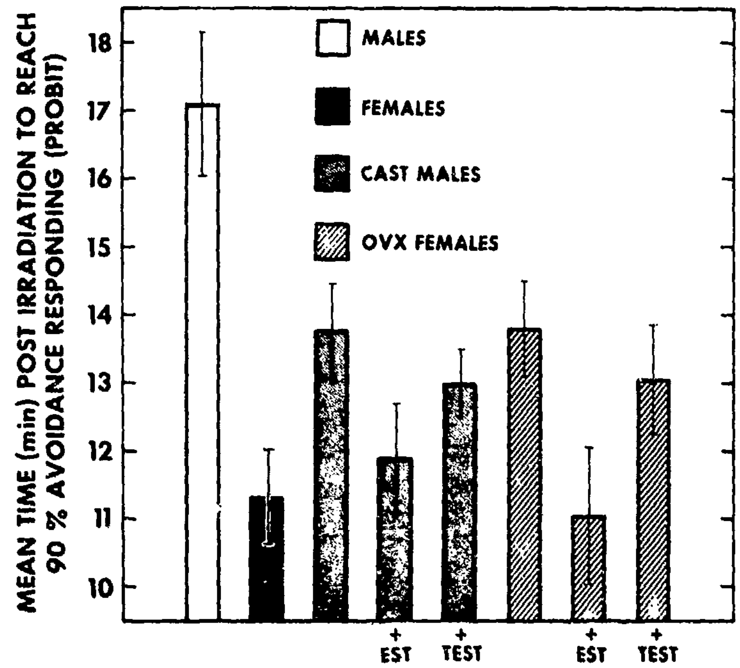

FIG. 6. Interpolated recovery time data from the fitted probit curve. The difference noted between intact male rats and female rats after irradiation was eliminated by gonadectomy (OVX = ovariectomy, CAST = castration). Testosterone (TEST) treatment only slightly decreased this time to recover. Estradiol (EST) allowed neutered animals to recover most rapidly. Variance indicators are the standard error of the mean.

test was then used to compare these changes in blood pressure in males and females. The difference between the two groups was highly significant (P = 0.001). Clearly the blood pressure changes that males experienced (hypotension) postirradiation were quite dissimilar from those of the female (hypertension).

It is interesting to note, however, that the baseline blood pressures for males and females also differed significantly (t test, $P < 0.01$). Female baseline pressure was consistently lower than that of the males. Thus, while there was a trend for female absolute pressure measurements taken postirradiation to be above those of the males, it was not a significant one ($P > 0.05$) (see Fig. 4).

It is generally assumed that the *drop* in male blood pressure is a critical factor in accounting for behavioral performance decrements (*7*). Since, in the present study, the absolute postirradiation blood pressures for both sexes were similar, the pressure fluctuations observed within individuals may well make a greater contribution toward an explanation of the deficits on the avoidance task.

Systemic Hormone-Manipulation Experiments

Avoidance Task

Control-injected castrated males and ovariectomized females behaved quite similarly on the shock-avoidance task after irradiation. A Friedman two-way analysis of variance (*37*) indicates no difference between oil-injected gonadectomized males and females at 2.5 min ($P > 0.50$), 7.5 min ($P > 0.50$), and 17.5 min ($P > 0.70$) postirradiation (see Fig. 5). In addition, when the data are fit to a probit curve (see data points in Fig. 5 for an idea of goodness of fit) and the time to reach 90% avoidance responding is computed, it can be determined that both

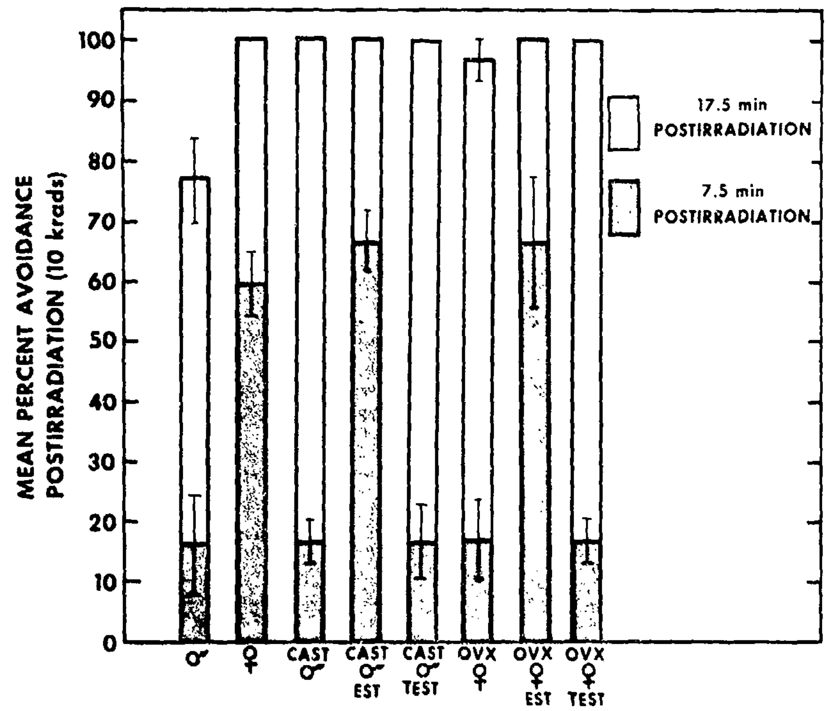

FIG. 7. Mean percentage avoidance in two time periods postirradiation. Intact females and neutered animals (OVX = ovariectomy, CAST = castration) that received estogens (EST) performed the avoidance task better in the early stages of the ETI (5.0–7.5 min postirradiation) than did oil-injected gonadectomized rats, those treated with testosterone (TEST), or intact males. Late in the ETI (15.0–17.5 min postirradiation), all neutered animals performed similarly. Variance indicators are the standard error of the mean.

groups of neutered animals that received control injections recovered in about the same time after irradiation ($P > 0.50$, Friedman two-way analysis of variance) (see Fig. 6). It is interesting to compare these gonadectomized animals to intact rats that were run as a replication of the sex dichtomy experiments. As can be seen in Figs. 5 and 6, gonadectomized rats performed at a 90% avoidance level after a time period that lay between those of intact males and females. However, these neutered animals did not differ significantly from intact females on this measure ($P > 0.05$, Kruskal–Wallis). Surprisingly, in the early stages of the ETI (i.e., 5.0–7.5 min), the severe performance decrement observed in neutered rats was similar to those of the intact male ($P > 0.05$, Kruskal–Wallis) and different from those of the intact female ($P < 0.05$, Kruskal–Wallis). However, in the later states of the ETI (15.0–17.5 min postirradiation), control-injected neutered rats did not differ from the intact female ($P > 0.05$, Kruskal–Wallis) and were significantly different from the intact males ($P < 0.05$. Kruskal–Wallis) (see Fig. 7). Thus, oil-injected castrated males and ovariectomized females recovered in a time more closely resembling that of the intact female.

Since gonadectomized male and female responses were not significantly different, hormone treatment comparisons were made on the combined data using a Kruskal–Wallis one-way analysis of variance and multiple comparisons ($\alpha = 0.05$). Figure 8 illustrates the postirradiation avoidance response of gonadectomized animals that received sham hormone injections and the same response of the neutered rats that received testosterone. Clearly, testosterone had little

or no effect on performance of the avoidance task, and no statistically significant difference is achieved ($P > 0.05$) between the hormone-treated and nontreated gonadectomized rats at 2.5, 7.5, or 17.5 min after exposure. Likewise, interpolation of the time to reach 90% (using probit curve fitting) indicated that testosterone neither lengthened nor shortened this period ($P > 0.05$).

Estradiol treatment, on the other hand, produced a compelling (and statistically significant, $P < 0.05$) improvement in the gonadectomized animals' ability to perform in the first 2.5 min immediately following exposure (see Fig. 9). This enhanced rate of avoidance responding is also evident in the 5- to 7.5-min period ($P < 0.05$). Late in the ETI (15- to 17.5-min period postirradiation), this difference disappeared as all animals (except the intact males) seemed to quickly approach asymptote (see Figs. 6 and 7). Still (as seen in Fig. 6), the Kruskal–Wallis test reveals that estradiol-treated neutered rats achieved a 90% avoidance rate of responding significantly ($P < 0.05$) more quickly than the sham-injected gonadectomized animals.

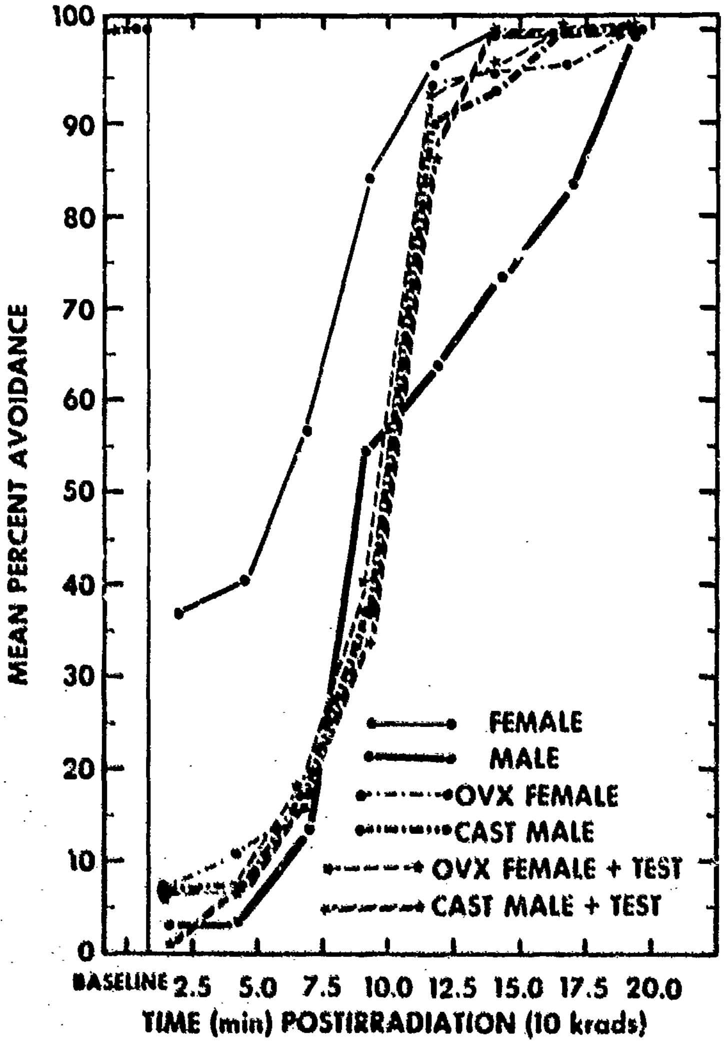

FIG. 8. Mean percentage avoidance as a function of time postirradiation. Gonadectomized rats that received control injections (OVX = ovariectomy, CAST = castration) performed like those injected with testosterone (TEST). These rats suffered initial decrements as severe as intact males but recovered quickly, like intact females.

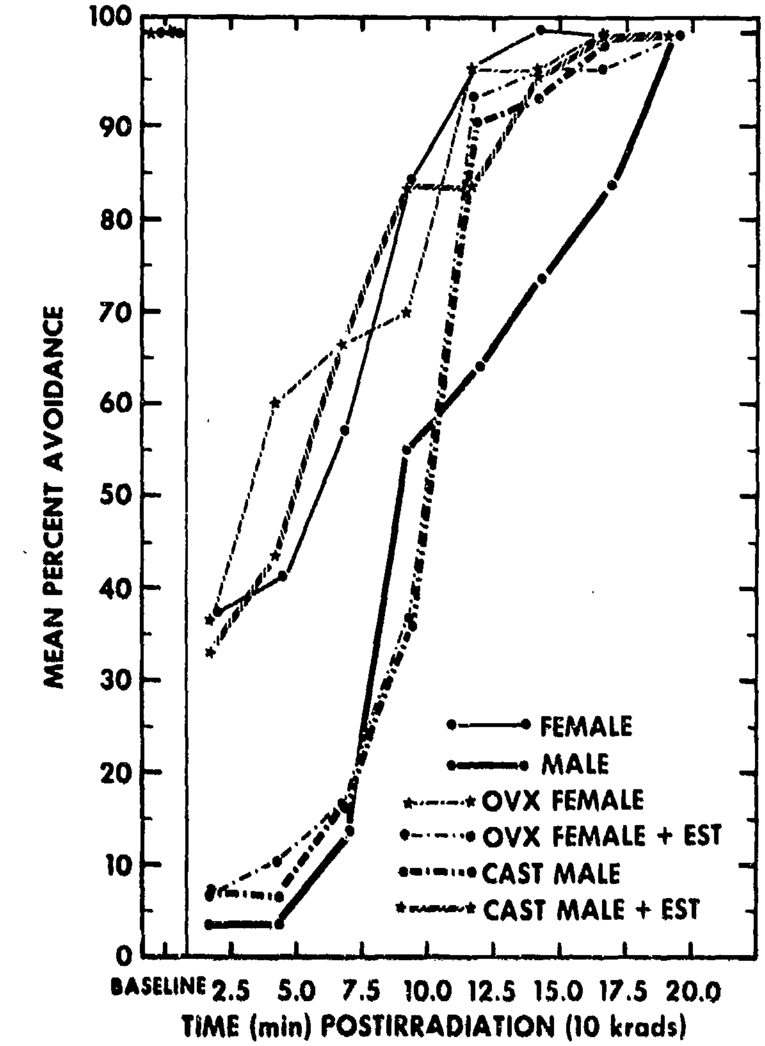

FIG. 9. Mean percentage avoidance as a function of time postirradiation. After irradiation, gonadectomized rats treated with estradiol (EST) outperformed oil-injected ovariectomized (OVX) female and castrated (CAST) male rats. These hormone-treated neutered animals behaved similarly to intact females and differently from intact males.

Gonadectomized animals that had received estogens did not show as profound an initial performance decrement and recovered more quickly than either sham-injected or testosterone-injected neutered rats. Generally their behavioral response was strikingly similar to that of the intact females. Comparisons between the intact females and gonadectomized rats of both sexes that received estradiol revealed no significant differences ($P > 0.05$, Kruskal–Wallis) at any time period (i.e., 2.5, 7.5, 17.5 min) postirradiation or in the time to reach 90% avoidance responding.

Blood Pressure

After irradiation, gonadectomized rats of both sexes experienced a drop in blood pressure (see Fig. 10) which was not altered by testosterone. However, estradiol treatment in castrated males and ovariectomized females caused a dramatic rise in pressure which resembled that of the intact females (see section on sex dichotomy experiments).

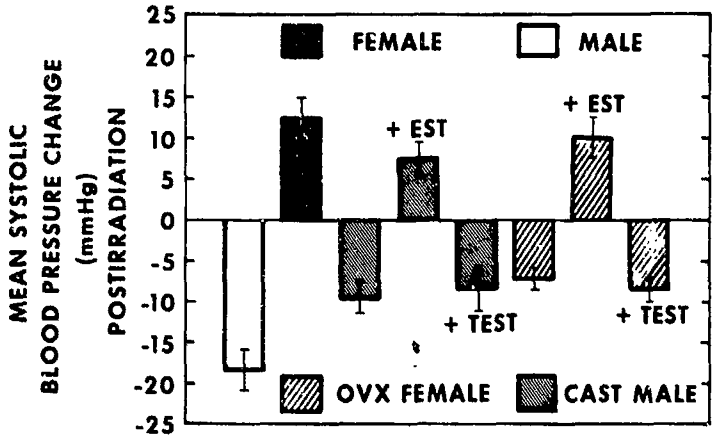

FIG. 10. Postirradiation blood pressure changes. Sex differences in the blood pressure response to radiation are eliminated by gonadectomy (OVX = ovariectomy, CAST = castration). While testosterone (TEST) does not alter the postirradiation hypotension associated with gonadectomy, estradiol (EST) reverses it, producing a rise in blood pressure. Blood pressure "change" is post-irradiation systolic pressure minus baseline systolic pressure. Variance indicators are the standard error of the mean. Data from intact animals of the sex dichotomy experiment are reproduced here to allow comparison.

In order to achieve a normal distribution, all blood pressure scores were converted to logarithmic form and then the mean difference between the post-irradiation and baseline measures was computed for each animal. A two-way analysis of variance and subsequent t test revealed no significant difference ($P = 0.650$) between the blood pressure changes of castrated males and ovariectomized females within any of the three treatment groups. That is, both sexes of neutered animals appeared to be similar if they received similar hormone

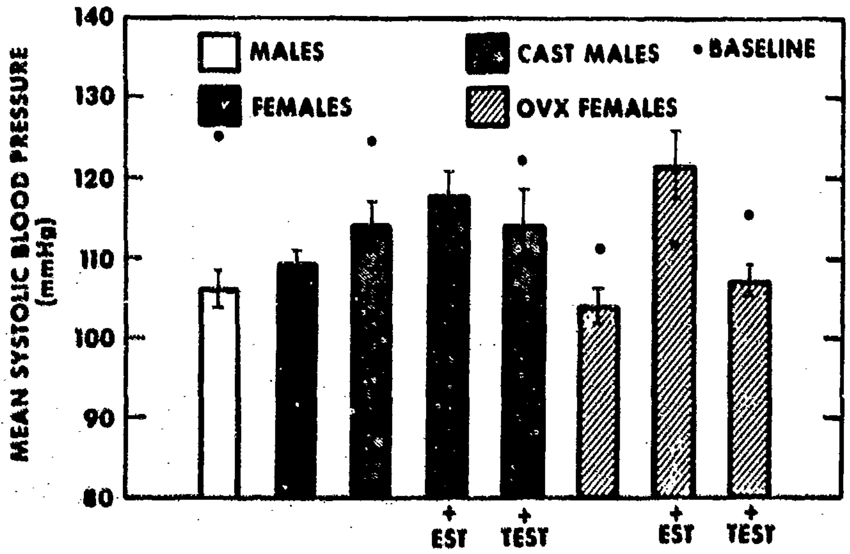

FIG. 11. Baseline and postirradiation systolic blood pressure measures. In contrast to blood pressure "changes" (see Fig. 10), actual postirradiation blood pressure levels and baseline blood pressures of gonadectomized (OVX = ovariectomy, CAST = castration) rats are similar regardless of sex or hormone treatment (TEST = testosterone, EST = estradiol). Responses of intact animals are reproduced from the sex dichotomy experiment. Variance indicators are the standard error of the mean.

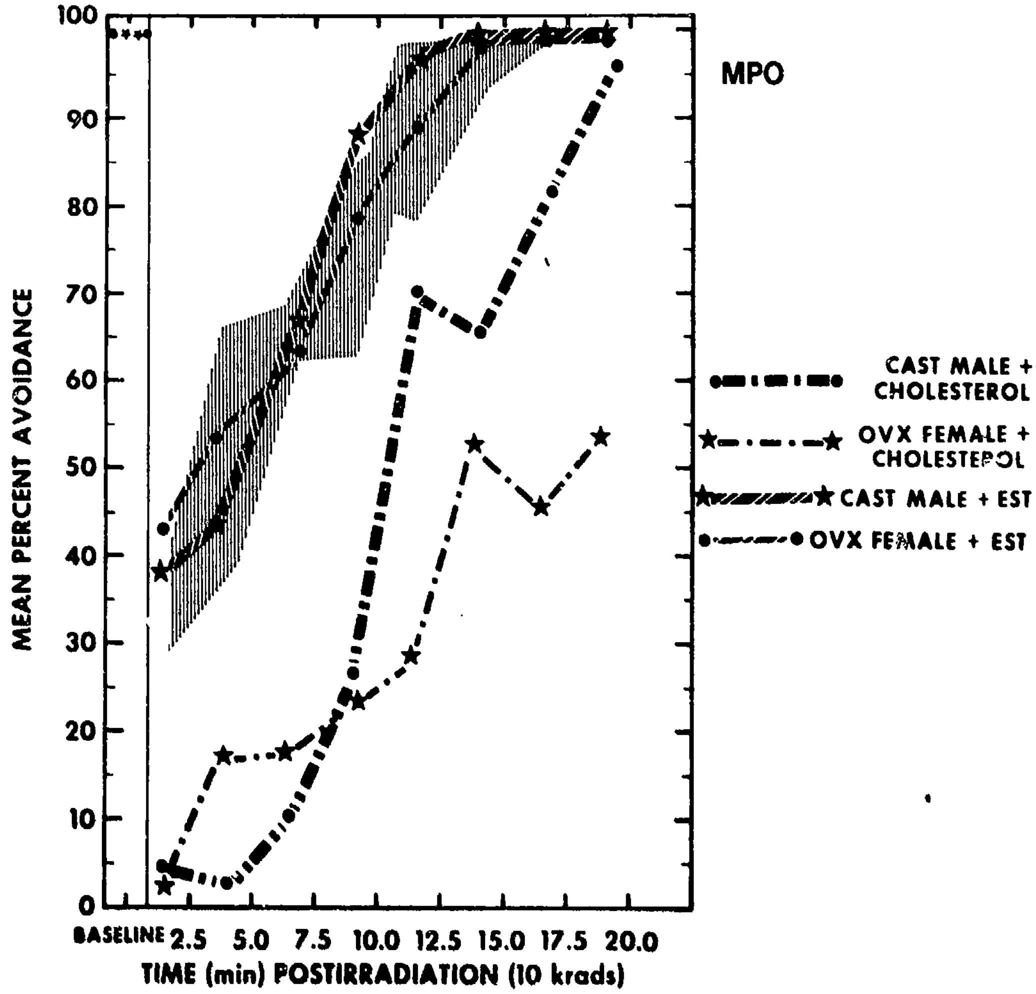

FIG. 12. Mean percentage avoidance as a function of time. After irradiation, gonadectomized rats (CAST = castrated male, OVX = ovariectomized female) with estradiol (EST) implants in the nucleus preopticus medialis (MPO) perform in a manner superior to those with control (Cholesterol) implants. The shaded area represents the combined standard errors of the mean of rats treated with systemic sc injections of estradiol (from Fig. 9).

treatments. Gonadectomy attenuated the profound intact-male hypotension postirradiation (see Fig. 10) observed previously. However, the intact female hypertension response was reversed by ovariectomy, which produced a mild drop in blood pressure. Testosterone did little to alter these effects as both groups of male and female gonadectomized animals showed a drop in blood pressure similar to the oil-injected neutered rats. An analysis of variance and t tests among treatments indicated no significant difference between the oil-injected and testosterone-treated groups ($P = 0.936$). Estradiol injections, however, produced a compelling hypertension after irradiation in gonadectomized rats of both sexes. Blood pressure changes of these animals were significantly different from those of the sham-injected group ($P < 0.001$) as well as the testosterone-treated group ($P < 0.001$).

A two-way analysis of variance was performed on the log-transformed baseline measures as well as on the mean of the logarithm of the postirradiation blood pressure scores (see Fig. 11). Neither comparisons between sexes nor comparisons

between treatments were found to achieve an α of 0.05. That is, different hormone therapies influenced postirradiation blood pressure *changes* but had no effect on *absolute* systolic pressure levels before or after irradiation.

Intracranial Hormone-Manipulation Experiments

Avoidance Task

In this study it was not possible to use the same statistical methods as in the previous experiments. Probit curve analysis was difficult due to the severe postirradiation deficits and lack of recovery seen in the MA treatment groups. The data in these groups did not fit a normal distribution, and the intra-group variance of several measures was zero. This suggested a noncontinuous distribution which made the use of even nonparametric statistics of questionable validity. Although it was difficult to determine the probability of differences between these groups, the use of summary data gave a clear picture of the results of this study: Implantation of estradiol crystals in the MPO improved the subject's postirradia-

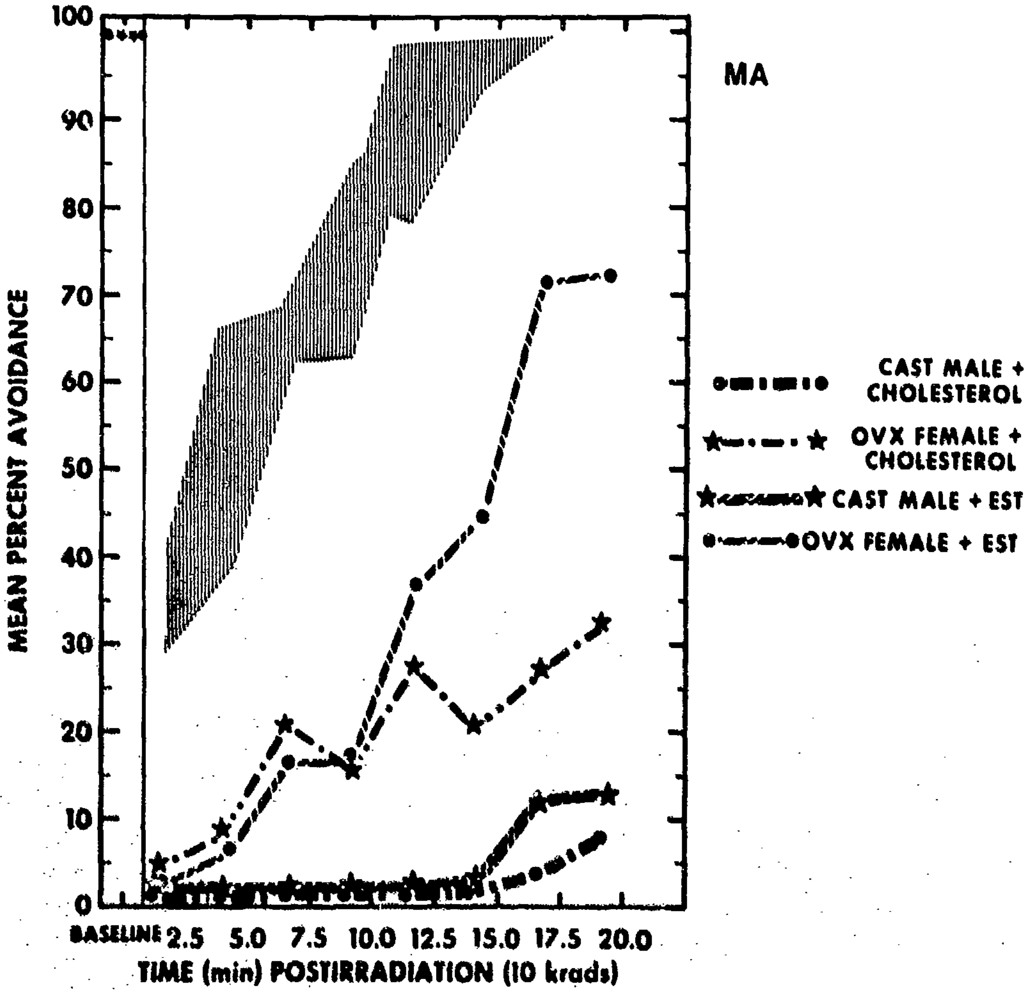

FIG. 13. Mean percentage avoidance as a function of time postirradiation. Estradiol (EST) in the nucleus amygdaloideus medialis (MA) does not enhance the postirradiation avoidance performance of gonadectomized rats (CAST = castrated males, OVX = ovariectomized females) over those levels achieved with control cholesterol implants. The shaded area represents the standard errors of the mean of rats treated with systemic sc injections of estradiol (from Fig. 9).

TABLE II

Percentage of Subjects[a] That Recovered[b] Their Ability to Avoid Foot Shock after Irradiation

	Nucleus preopticus medialis		*Nucleus amygdaloideus medialis*	
	Estradiol (%)	*Cholesterol* (%)	*Estradiol* (%)	*Cholesterol* (%)
Castrated male	100	83	0	0
Ovariectomized female	100	50	50	16

[a] $n = 6$.

[b] Recovery is defined as avoiding shock on at least 90% of the trials in any 2.5-min period in the first 20 min postirradiation.

tion avoidance performance in a fashion similar to that seen after systemic injections (see Fig. 12).

The percentage avoidance for each 25-min period (five trials) was computed for each animal, and then the average scores of each group were plotted (Figs. 12 and 13). Figure 12 illustrates the benefits of estrogens in the MPO. Gonadectomized rats with control cholesterol implants in the MPO suffered severe avoidance-performance decrements in the first 2.5 min postirradiation. None of these animals were able to perform a single avoidance in this time period (see Table III). By way of contrast, in this same initial period, the performance of the individual rats treated with estradiol never dropped below 20%. Thus there is no overlap between the scores of estrogen-treated and control animals. The shaded portion of Fig. 12 represents the combined standard errors of the means for gonadectomized rats of both sexes that received systemic injections of estradiol (from systemic hormone-manipulation experiment, Fig. 9). It should be noted that the avoidance recovery curves of the estrogen–MPO group in the present experiment fall almost exclusively within this range. Thus estrogen's action on the CNS produced behavioral effects postirradiation that closely resemble those derived from systemic injections of this steroid.

MPO–cholesterol implantation seems to interact with irradiation to produce some deficits in avoidance in the ovariectomized female that were not observed in the castrated males in the present study or in the control-injected gonadectomized rats of the systemic-hormone experiments. Of these six MPO–cholesterol animals, only three recovered to the 100% baseline avoidance-performance level during the 20 min they were observed. Generally, subjects that had received estradiol in the MPO recovered (90% avoidance) more often (Table II) and achieved higher percentage avoidance scores (see Table III) than those that had been implanted with cholesterol. Also, if one compares the animals of both groups (estrogen–MPO and cholesterol–MPO) that did recover to 100% avoidance, it can be determined that rats treated with intracranial estradiol recovered to this preirradiation baseline level more quickly than did the rats with the control implants ($P < 0.05$, Mann–Whitney U) (*37*).

In regard to the severity of initial avoidance decrement, irradiated gonadectomized rats with estrogens in the MPO were far superior to those rats that received

estrogens in the MA (see Table III). None of the 12 rats implanted with estrogens in the MA performed a single avoidance in the first 2.5 min (lowest point) after exposure. On this measure, these animals were identical to the rats with control (cholesterol) implants in the MA (see Fig. 13 and Table III). Full recovery to 100% avoidance performance rarely (3 rats out of 12) occurred in subjects with estrogens in the MA and never occurred (0 rats out of 12) in subjects with cholesterol in this brain region. This is in sharp contrast to rats with MPO–estradiol implants which always recover to 100% (Table II; see also Table III, which shows this general recovery difference).

The noncongruence of the shaded area and the data curves of Fig. 13 illustrates the dissimilarity between the detrimental effects of estradiol in the MA and the benefits associated with systemic estradiol. Interestingly, the discriminating factor that separates the animals with MPO implants is the steroid used; i.e., rats with estrogens performed in a superior fashion if compared to those with cholesterol implants. In the MA, however, neutered animals of the same sex tended to show similar responses independent of the hormonal treatment they experienced. Tables II and III indicate that, although none of the animals with the MA implants recovered from the radiation-induced ETI very successfully, ovariectomized females were superior in this regard.

Blood Pressure

The irradiation of gonadectomized rats with estradiol in the MPO produces a mild hypertension similar to that seen in both intact female rats and in neutered rats that received systemic injections of estrogens. Implantation of estradiol into the MA did not produce this hypertension response.

TABLE III

Highest[a] and Lowest[b] Avoidance Scores[c] Achieved Postirradiation[d]

	Nucleus preopticus medialis		*Nucleus amygdaloideus medialis*	
	Estradiol	*Cholesterol*	*Estradiol*	*Cholesterol*
Castrated male	100 (0) / 30.83 (8.41)	96.96 (3.33) / 0 (0)	13.33 (13.33) / 0 (0)	10 (6.83) / 0 (0)
Ovariectomized female	100 (0) / 43.33 (13.08)	53.33 (21.08) / 0 (0)	76.67 (12.92) / 0 (0)	30 (19.15) / 3.33 (3.33)

[a] Upper division.

[b] Lower division; these scores were always during the initial (0- to 2.5-min) period postirradiation.

[c] Group-mean percentage-avoidance scores. SEM shown in parentheses.

[d] In any of eight 2.5-min time periods.

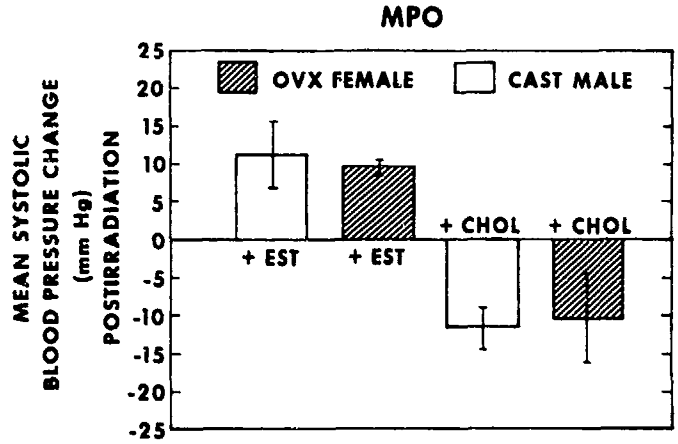

FIG. 14. Gonadectomized rats (CAST = castrated males, OVX = ovariectomized females) with estradiol (EST) in the nucleus preopticus medialis (MPO) exhibit an increase (postirradiation pressure minus preirradiation baseline) in systolic blood pressure after irradiation. Rats injected with cholesterol (CHOL) are hypotensive in this same time period. Variance measures are the standard error of the mean.

The average systolic blood pressure after irradiation was computed for each animal, and the baseline blood pressure was subtracted from this value to derive a change statistic. The mean of these changes was determined for each group of gonadectomized rats (see Figs. 14 and 15).

Irradiation caused an increase in blood pressure in ovariectomized females and castrated males with estradiol in their MPOs. This mild hypertension was significantly different ($P < 0.01$, Mann–Whitney) (*37*) from the hypotension observed in the gonadectomized control groups which received cholesterol in the MPO (see Fig. 14). The postirradiation hypertensive response observed in the rats implanted with estradiol in the MPO was also significantly different from the hypotension of the neutered rats with estrogens in the MA ($P < 0.05$, Mann–Whitney U).

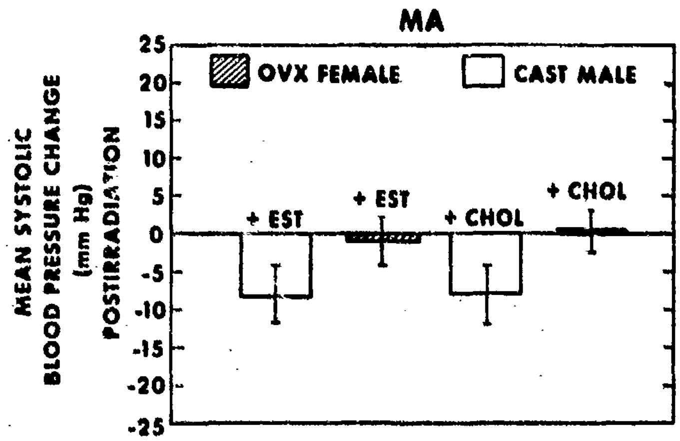

FIG. 15. Castrated male rats (CAST) with either estradiol (EST) or cholesterol (CHOL) implants in the amygdaloideus medialis (MA) exhibit a hypotension postirradiation (postirradiation pressure minus preirradiation baseline). The blood pressure of ovariectomized females (OVX) changes little in this same time period. Variance measures are the standard error of the mean.

Estradiol had no significant effect on the systolic blood pressure of irradiated neutered rats if it had been placed in the MA (Fig. 15). Castrated male rats were hypotensive regardless of the steroid crystals present in the MA ($P > 0.05$, Mann–Whitney U). A comparison of the blood pressures of ovariectomized females with estradiol and those with cholesterol in the MA also revealed no significant difference ($P > 0.05$, Mann–Whitney U). As was the case in the avoidance measure of the last experiment, gonadectomized animals of common sex which had steroids implanted in the MA appeared to have similar blood pressure responses regardless of the steroid present. The blood pressure of both groups (MA–estradiol and MA–cholesterol implants) of ovariectomized females was generally unaltered by irradiation while estrogen-implanted and cholesterol-implanted castrated males were similarly hypotensive.

It must be noted, however, that in the case of the MPO implants, the blood pressures of all subjects in each group were consistent; i.e., all showed hypertension (in the case of the estradiol implants) or all exhibited hypotension (in the case of cholesterol implants). There was no such consistency in the MA groups. In the ovariectomized female–cholesterol group, for example, half of the rats were hypertensive postirradiation and the other half were hypotensive. Therefore, conclusions about MA-implanted rats (particularly the ovariectomized female groups) must be somewhat tentative.

DISCUSSION

The sex dichotomy study clearly demonstrated that female rats of the Sprague–Dawley strain were more resistant than males to avoidance-performance decrements produced by a large dose of ionizing radiation. In addition, blood pressure responses of the two sexes differed significantly. Females did not show the profound hypotension typically associated with the male and correlated with behavioral incapacitation.

Sex differences have, of course, been noted previously on various behavioral tasks. It is of particular relevance to the present investigation that female rats acquire avoidance behaviors more rapidly than do males (*17*). Casual observation in our own laboratory confirmed this finding. This difference is apparently not related to a disparity in body weight, age, or reproductive experience. Rather, Beatty and Beatty (*17*) suggest that the female superiority may be due to a greater sensitivity to electric shock. This is supported by their finding that females have slightly lower flinch and jump thresholds than do males. However, since the level of current used in the present study was well above threshold, it is questionable whether these data bear directly on the experiment reported here. In addition, the mechanisms that underlie acquisition of an avoidance response may not necessarily be the same ones that mediate the performance of such a response after some acquisition criterion has been met.

Thorp and Young (*30*) have demonstrated that, after irradiation, head-shielded female monkeys perform better on a simultaneous visual discrimination task than do shielded males. However, performance of unshielded males was significantly better than that of unshielded females. The difference between this latter result and the present finding may be a function of the species used or the

different tasks used. It has been suggested by Broverman *et al.* (*45*) that females excel on simple, overlearned, perceptual motor tasks and males do better on more complex tasks requiring an inhibition of immediate responses to obvious stimulus attributes in favor of responses to less obvious stimulus attributes. Thus it may be the case that the postirradiation female superiority described here might not be as easily demonstrated on more "cognitive" tasks. It should also be noted that the present demonstration of postirradiation male hypotension and female hypertension may apply only to systolic blood pressures as monitored peripherally by the tail-occlusion method. Others (*16*) have shown that blood pressure at a peripheral site can be decreased at a radiation dose level that does not affect pressure determined by aortic intubation. There exists, however, a linear relationship between systolic pressure recorded by these two methods. Still, because the 10 krad used in the present study is well above the apparent threshold dose needed to achieve a hypotension in males, it may be the case that aortic pressure (although it was not measured here) may have also declined in these animals.

The systemic hormone-replacement experiments showed that the ETI of control-injected gonadectomized rats of both sexes was of short duration ($\simeq$15 min) and approximated that of the intact female. However, initial performance decrements were as severe as those experienced by intact males. Testosterone injections did not alter the ETI of neutered animals, but estradiol produced "feminine" avoidance responses in these rats. Clearly estradiol had a dramatic beneficial effect on the behavioral capacity of the irradiated rat.

A review of Figs. 5 through 9 reveals that there may be at least two general avoidance-performance measures of interest: ETI duration and ETI intensity. Overall, one might say that gonadectomy had favorable behavioral effects (ETI shortening) on males and unfavorable behavioral effects (more intense incapacitation) on females. Since estradiol injections in ovariectomized females produced an effect that resembles that of the intact female in both severity of initial decrement and time to recover, one may be justified in assuming that the profound initial decrement in sham-injected spayed rats was due to the lack of this hormone. Similarity, only the presence of estradiol (not testosterone) enhanced early performance of castrated males.

Inspection of the slopes of the recovery curves for sham-injected gonadectomized rats reveals a salient characteristic of the ETI-shortening effect. The fact that these animals recovered at the same time as intact females but suffered greater initial performance decrements suggests that gonadectomy has actually shortened the ETI by increasing the *rate of recovery* as opposed to merely decreasing the time to reach some recovery criterion that may be dependent on other factors: e.g., degree of the initial impairment. Estrogens may play little part in determining duration of the ETI since animals with presumably low estrogen levels (castrated males and ovariectomized females) recovered quite quickly without benefit of that gonadal hormone. While estrogens appear to play the key role in the female's superior initial performance and blood pressure responses after irradiation, the hypothesis of a beneficial effect of androgen absence cannot be entirely discounted. The adrenal glands constitute a significant source of androgen that is not eliminated by loss of the gonads (*46*). Thus the circulating levels of

testosterone in castrated males, while certainly diminished, may be sufficient to allow the behavioral and blood pressure decrements hypothesized in the introduction. This notion is consistent with the fact that castration allows an ETI of shorter duration than that seen in the intact male. However, this speculation is not supported by (a) the fact that castration failed to enhance the initial avoidance performance or alter the blood pressure response of irradiated male rats, or by (b) the fact that exogenous testosterone did not produce a longer ETI in castrated males. Neutered animals recovered in about the same amount of time independently of whether they received testosterone.

Other explanations for the fact that castrated male avoidance-recovery time approximates that of the intact female include the possibility that castration allows either the removal or disinhibition of some physiological factor (other than testosterone) that normally prolongs the ETI. Alternatively, the long recovery time of the intact male could reflect an effect of chronic endogenous androgens which are not present in gonadectomized rats.

Like the various studies reporting estrogen's long-term radioprotective capacities (*12–15*), the present investigation confirms the behavioral benefits of circulating estrogens in *acute*-radiation behavioral phenomena.

Postirradiation blood pressure changes in gonadectomized rats followed a pattern similar to that of the behavioral measures. Gonad removal attenuated somewhat the profound hypotension observed in intact males. The drop in blood pressure showed by spayed females contrasts with the hypertension of the intact female. Testosterone had no effect on the postirradiation blood pressure decline of neutered rats, but estradiol caused a significant increase in blood pressure in these animals. These data are consistent with the work of Altura (*21*) and Von Eiff and Piekarski (*47*), whose data argue that estrogens protect animals against circulatory stress reactions.

Unpublished observations from this laboratory reveal that the behavior and blood pressure of irradiated intact males and females cannot be manipulated by systemic injections of gonadal hormones. In hormone-supplement paradigms identical to those reported here, intact males treated with estradiol benzoate or testosterone propionate consistently exhibited a severe ETI and drops in systolic blood pressure that were similar to those of the noninjected controls. Likewise, intact irradiated females injected with either of the steroids always showed avoidance and blood pressure responses similar to those of the untreated females. As has been shown in other paradigms (*48*, *49*), exogenous gonadal hormones impact less dramatically on the behavior and physiology of adult intact animals then they do on neonatally gonadectomized subjects.

Previous investigators have also demonstrated that it is possible to produce female sexual behaviors by implanting estrogens in the hypothalamus (*50*). The present study suggests that estradiol implants in the MPO are sufficient to produce the less severe "female" ETI response to a supralethal dose of ionizing radiation. These behavioral and blood pressure benefits were similar to those observed in irradiated animals after *systemic* injections of estradiol. Estrogen implants in the MA did not offer these advantages. The results suggest a primary CNS mechanism behind estradiol's radioprotective effects. More specifically,

the MPO appears to contain the cells critical to the avoidance and blood pressure benefits derived from estradiol exposure. It is possible to localize estrogen's site of action at the MPO since, in the time period used in the present experiment, 98% of the injected steroid has been shown to remain at the point of implantation (*51*).

Despite the fact that estrogen-containing neurons are present in both MPO and MA (*33–36*), there are anatomical differences between the two areas that could provide a basis for their apparently differing functional roles in radioprotection. While 13% of the cells in the MPO concentrate estrogen, 30% of the cells in the MA do so. The absolute number of estrogen-containing cells in the MA is also more than double those in the MPO (*52*). In addition, although both brain regions have been implicated in motivation (*53*) and have common neural interconnections (*54*), there exist known functional differences between the two areas. For example, lesions of the MPO sharply attenuate estradiol-induced locomotor activity, whereas MA lesions fail to interfere with this response (*55*).

Other investigators have reported decrements in avoidance performance after amygdala lesions (see review by Thompson, Ref. (*56*)). Since insertion of the injection cannula and crystalline steroid causes some brain damage, this could explain part of the severe behavioral decrements seen postirradiation in animals with MA implants. However, this notion is not supported by the facts that animals with MA implants displayed a consistent sex dichotomy and those with MPO implants displayed a dichotomy based on the steroid used. If the severe ETI were merely due to a lesion common to all subjects, one would probably not expect to observe group differences such as those.

The sex dichotomy observed in both the performance and blood pressure responses of irradiated, gonadectomized rats with MA implants raises some questions about the cells present in that brain area. A nucleus in the hypothalamus has been identified as being sexually dimorphic in that the nuclear volume of this region is significantly greater in the male than in the female. This nucleus has been suggested as a possible morphological basis for sex differentiation of brain function (*57*). Perhaps the radiosensitivity of cells or neural circuits present or adjacent to the ovariectomized female MA is not identical to the radiosensitivity of the MA cells present in the castrated male rat. Alternatively, it may be the case that the effect of estrogen exposure at the MA produces sex-dependent morphological or physiological changes at this site. Some evidence is presently available to suggest that prepuberal ovariectomy can alter CNS morphology and that estrogen feedback may be critical to nuclear development (*58*).

To a degree, the present study localizes estradiol's apparent radioprotective action at the MPO. However, the fact that an intracranial estrogen implantation can produce an attenuated ETI does not necessarily preclude the possibility of peripheral involvement in the syndrome. Nor does this study exclude the possibility that extrahypothalamic and/or nonsteroidal neurochemical events may interact with estradiol to produce the radioprotection reported here. These interactions could take many forms. As was stated in the Introduction, estrogens may influence the excretion of histamines (*10, 11*) which, in turn, have been implicated in the etiology of the ETI syndrome (*5, 9*). In addition, estradiol's

known influence on the catecholamine systems (*17*, *59*) [considered important in the learning and maintenance of avoidance tasks, Refs. (*60–67*)] and interactions with the anterior pituitary (*68–71*) and peripheral endocrine organs (*72*) suggest a variety of hypotheses concerning potential substrates of estrogen radioprotection.

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A sufficiently large and rapid dose of ionizing radiation produces an immediate but transient behavioral incapacitation. Acute hypotention often accompanies the disorder. Although the etiology of this syndrome is unclear, it has been suggested that an increase [illegible] histamine excretion contributes to it. Since histamine is known to interact with the [illegible]ocrine system and since estrogens have been shown to prolong the life of animals [illegible]d to potentially lethal doses of radiation, it was also hypothesized that females

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20. ABSTRACT (continued)

might be relatively less affected by an acute, large dose of ionizing radiation. Male and female rats were trained on an avoidance task, irradiated, and then retested. Females showed a less severe decrement after radiation exposure than males. Likewise, females did not suffer the severe hypotension normally associated with male radiogenic early transient incapacitation (ETI); rather, an acute hypertension was produced in females. A second series of experiments revealed that differences in male and female radiation response were eliminated by gonadectomy. Systemic estradiol injections produced strikingly feminine (i.e., superior) postirradiation avoidance responses as well as hypertension in neutered rats. Testosterone injections had no effect on either measure. Central nervous system alterations have been correlated with the ETI. Therefore, final experiments sought a possible central locus of the action of estradiol. It was found that exposure of the nucleus preopticus medialis to estrogens produces postirradiation benefits in avoidance performance and blood pressure similar to those seen after systemic estradiol treatments. Nucleus amygdaloideus medialis implants produced no such benefits.